Lentiviral vectors for immunotherapy of cancer

We propose to create an efficient protocol for expansion of anti-tumor epitope-specific T cells in vitro and in vivo that will not be biased toward immunodominant peptides. We shall take advantage of the specialized phenotype of dendritic cells (DCs). Dendritic cells are monocyte (or bone marrow)-dereived professional antigen-presenting cells (APCs) that can prime resting and native T lymphocytes and generate memory responses without additional exogenous adjuvant. We will use an artifical strategy to load MHC class I molecules with selected CTL epitopes.

Peptide sequences derived from the prostate-specific membrane antigen (PSMA-P1 and PSMA-P2 peptides) and from the Epstein-Barr virus (EBV)-derived latent membrane protein (2NC peptide) have been fused by a peptide spacer to the N-terminal end of the b2-microglobulin. The selected peptides are T cell epitopes containing anchoring residues for binding to HLA-A2 (a common MHC allele in Caucasian populations).

A variation of this approach was also created by fusing the above peptides to the endoplasmic reticulum (ER) translocation signal from the adenovirus E3/19 K glycoprotein. The genes encoding for the fusion proteins (peptide-b2 or Er-peptide) have been cloned into lentiviral vectors, and subsequently used to transducer dendritic cell precursors. Human monocytes or CD34+ cells will be isolated from peripheral blood and induced (or not) to a mature DC phenotype.